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REVIEW

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Microglia: Activity-dependent regulators of neural circuits

Violeta Durán Laforet | **Dorothy P. Schafer**

Department of Neurobiology, Brudnick Neuropsychiatric Research Institute, University of Massachusetts Chan Medical School, Worcester, Massachusetts, USA

Correspondence
Dorothy P. Schafer, Department of Neurobiology, Brudnick Neuropsychiatric Research Institute, University of Massachusetts Chan Medical School, Worcester, MA 01605, USA. Email: dorothy.schafer@umassmed.edu.

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Abstract

It has been more than a century since Pío del Río-Hortega first characterized microglia in histological stains of brain tissue. Since then, significant advances have been made in understanding the role of these resident central nervous system (CNS) macrophages. In particular, it is now known that microglia can sense neural activity and modulate neuronal circuits accordingly. We review the mechanisms by which microglia detect changes in neural activity to then modulate synapse numbers in the developing and mature CNS. This includes responses to both spontaneous and experience-driven neural activity. We further discuss activity-dependent mechanisms by which microglia regulate synaptic function and neural circuit excitability. Together, our discussion provides a comprehensive review of the activity-dependent functions of microglia within neural circuits in the healthy CNS, and highlights exciting new open questions related to understanding more fully microglia as key components and regulators of neural circuits.

KEYWORDS
microglia, neural activity, synapses

INTRODUCTION

Over 100 years ago, the first in-depth characterization of microglia was performed by Pío del Río-Hortega, a student of Ramón y Cajal. From histological stains of brain tissue, Río-Hortega astutely recognized that microglia "show very clearly that their shape is mutable and conditional; that their protoplasm is capable of plasticity; and that they have, in short, a quality inherent to the migrant corpuscles, among which, in all probability, microglia must be included."[1] Fast forward to today and Río-Hortega's observations are being fully realized. Seminal two-photon live imaging studies demonstrated that microglia change their motility in vivo in response to neurotransmitters and changes in neural network activity.[2,3] This was further supported by data showing that microglia express neurotransmitter receptors and respond to neurotransmitters in vitro by modulating the production of immunological signals, including cytokines.[4,5] More recently, significant differences in microglial gene expression were identified following experience-dependent dampening of neural activity[6] and chemogenetic activation or inhibition of neurons.[7] Accumulating data indicate that microglia distinguish between increased and decreased activity and modify their phenotype accordingly. While there are many important emerging functions for microglia within neural circuits, we focus below on work showing key roles for microglia in sensing changes in neural activity and subsequently influencing synapse development, plasticity, and network activity within the healthy brain (Figure 1).

ACTIVITY-DEPENDENT DEVELOPMENTAL SYNAPTOGENESIS

Microglia are born from the embryonic yolk sac and enter neural circuits early in embryonic life.[8–10] In mice, this entry into the early embryonic central nervous system (CNS) occurs between ~E9—9.5.[8–10] Once in the CNS, microglia begin to colonize the brain,[11] where they adopt a unique resident microglia molecular signature[12] and become poised to play critical roles in shaping the development

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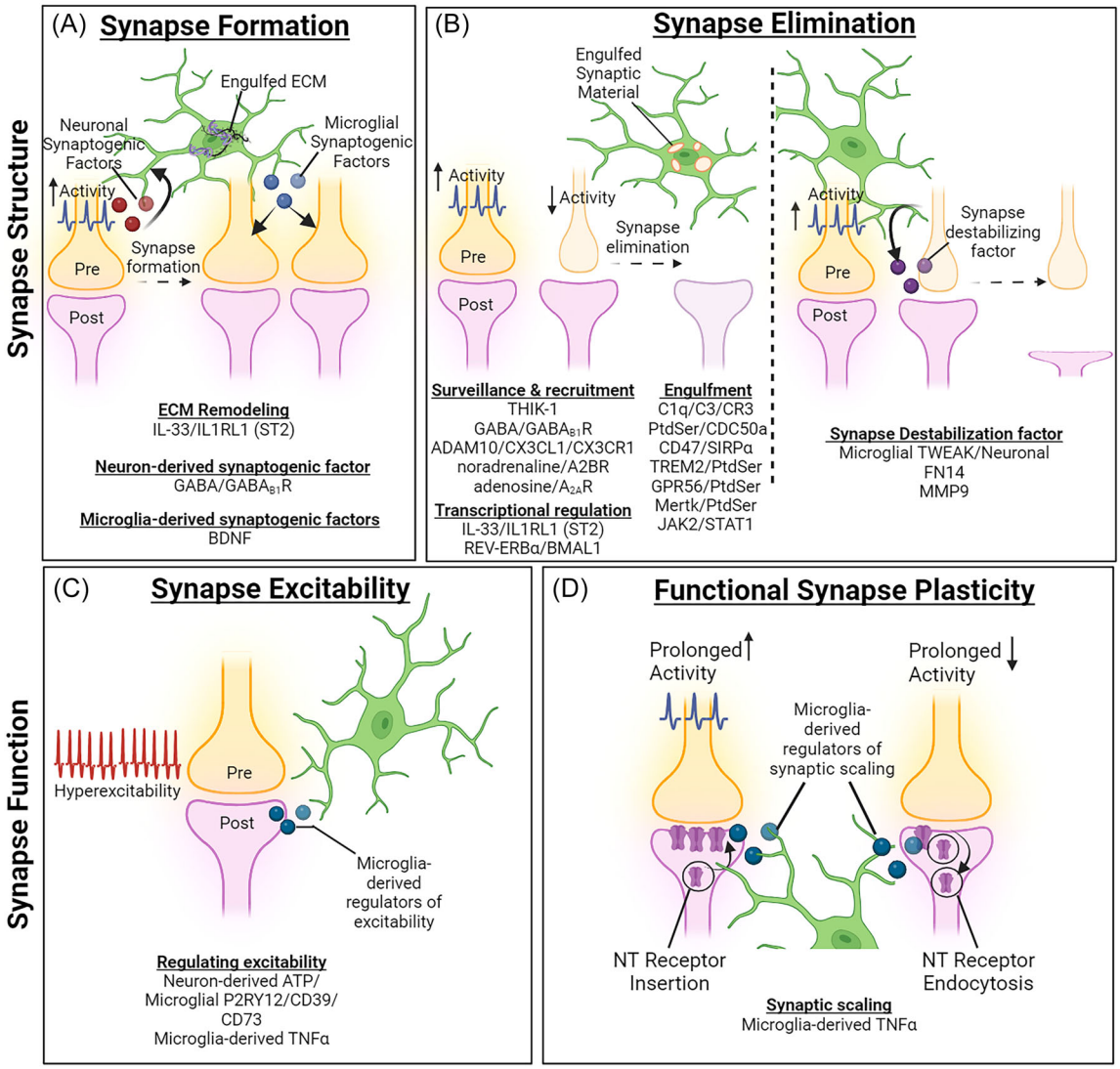

FIGURE 1 Summary of activity-dependent microglia functions within neural circuits. (A) Microglia can modulate synapse formation in response to a change in neuronal activity in response to neuron-derived molecules such as GABA and IL-33. GABA released by neurons binds $GABA_{B1}R$ on microglia to regulate chandelier cell synaptogenesis. Likewise, neuron-derived, and possibly astrocyte-derived, IL-33 binds the microglia-expressed receptor IL1RL1/ST2 to induce microglia to phagocytose ECM to accommodate new synapses. Microglia have also been proposed to release more BDNF during heightened activity in the motor cortex to induce new synapse formation. (B) Typically, a relative decrease in neuronal activity of a given synapse within a circuit triggers engulfment and elimination of that synaptic compartment by microglia (left panel). This microglia-mediated elimination of synapses involves multiple, different mechanisms that may act in concert, including modulation of surveillance and recruitment to synapses, transcriptional regulation of molecules regulating engulfment, and molecules that are critical to carry out engulfment of synaptic substrates; these mechanisms (listed) and can vary depending on the neuron subtype, brain region, and time of day. Microglia can also function to destabilize synapses in response to changes in neural activity (right panel). The most well characterized of these mechanisms is the interaction between neuron-expressed FN14 and TWEAK expressed on microglia, an interaction occurring in response to decreased activity in the visual system following dark rearing. (C, D) In addition to modulating synapse structure, microglia can modulate the functional connectivity of neurons in response to neuronal activity. (C) This has been most comprehensively shown in the context ATP (left) and cytokine-dependent modulation of synapses (right). Microglia can provide important feedback to block hyperexcitability by sensing ATP (left) released by hyperactive neurons and converting this ATP to adenosine via CD39 and CD73, which subsequently dampens activity. Microglia can also produce cytokines that modulate synaptic plasticity. (D) TNFα in the context of synaptic scaling whereby in response to prolonged increases (left) or decreases (right) in activity, microglia-derived TNFα induces the scaling down (left) or up (right), respectively, of postsynaptic neurotransmitter receptors. ADAM10, a disintegrin and metalloproteinase domain-containing protein 10; ADRB2, beta-2 adrenergic receptor; ATP, adenosine triphosphate; $A_{2A}R$, adenosine A_{2A} receptor; BDNF, brain-derived neurotrophic factor; BMAL1, brain and muscle ARNT-like protein 1; CD39, cluster of differentiation 39; CD47, cluster of differentiation 47, CD73, cluster of differentiation 73; CR3, complement receptor 3; CX3CL1, fractalkine; CX3CR1, fractalkine receptor; ECM, extracellular matrix; FN14, fibroblast growth factor-inducible 14; GABA, gamma-aminobutyric acid; $GABA_{B1}R$, GABA B1 receptor; GPR56, G-protein coupled receptor 56; IL-33, interleukin 33; IL1RL1 (or ST2), interleukin 1 receptor type 1; JAK2, janus kinase 2; MERTK, Mer tyrosine kinase; PtdSer, phosphatidyl serine; SIRPα, signal regulatory protein alpha; STAT1, signal transducer and activator of transcription 1; THIK-1, tandem pore domain halothane-inhibited potassium channel 1; TNFα,

(Continues)

FIGURE 1 (Continued)
tumor necrosis factor alpha; TREM2, triggering receptor expressed on myeloid cells 2; TWEAK, tumor necrosis factor-related weak inducer of apoptosis. Figure made with BioRender.

of neural circuits. As initial steps in the development of synaptic connectivity, axons must first grow out from the neuronal soma; then, synaptic connections must form. Demonstrating the importance of microglia in even the earliest stages of the CNS developmental process, genetic or pharmacological depletion of microglia in mouse embryos leads to defects in axonal outgrowth in the developing brain.[13,14] In the following sections, we review literature further supporting activity-dependent mechanisms by which microglia influence synapse formation in the developing brain (Figure 1A).

The initial work supporting a role for neural activity in microglia-mediated synaptogenesis in the developing brain was two-photon live imaging in the developing mouse somatosensory cortex that revealed elevated calcium in dendrites at sites of microglia contact.[15] At later imaging sessions, these contact sites had a higher probability of new dendritic spines, and ablation of microglia resulted in decreased spine numbers in the developing somatosensory cortex.[15] Similarly, another group showed by static imaging in fixed tissue that axon initial segments (AIS) contacted by microglia in layer 2/3 of the developing somatosensory cortex had greater numbers of GABAergic axo-axonic boutons from inhibitory chandelier cells, and that the GABAergic AIS boutons were reduced upon depletion of microglia.[16] This group went on to show that mice lacking $GABA_{B1}R$ demonstrated diminished microglia association with axons and decreased numbers of GABAergic axo-axonic boutons along the AIS.[16] These data suggest that microglia respond to GABAergic neurotransmission to modulate their association with the AIS, which subsequently facilitates chandelier cell inhibitory synapse formation.

Still, as the data in the above two studies are correlative, the molecular mechanism by which microglia execute these putative synaptogenic effects remains elusive. It is possible that this is not a synaptogenic effect, but rather an effect on synapse stabilization. Supporting the latter possibility, one of the groups above showed that microglia depletion in adulthood after developmental synaptogenesis similarly resulted in reduced GABAergic AIS boutons in the cortex.[16]

ACTIVITY-DEPENDENT SYNAPTOGENESIS IN THE ADULT CNS

In addition to regulation of synapse numbers in the developing brain, microglia have synaptogenic roles in the adult brain, based on work in the cortex,[17] hippocamus,[18] and olfactory bulb[19,20] (Figure 1A). Toward an activity-dependent mechanism, this was first suggested in the adult motor cortex.[17] Briefly, when animals were subjected to motor learning, new dendritic spines were observed by two-photon live imaging in the motor cortex of adult mice. When microglia were ablated, spinogenesis and motor learning were blocked.[17] The authors identified that microglia-derived brain-derived neurotrophic factor (BDNF) was critical for the generation of new spines following motor learning. As microglia express the least amount of BDNF of all resident CNS cell types[21] and BDNF is a secreted factor highly dependent on local concentration,[22] it remains to be determined how microglia-derived BDNF exerts such a potent effect. One possibility is that BDNF regulates intracellular signaling within microglia in a context-dependent manner (e.g., during learning vs. inflammation), which secondarily induces microglia to release other molecules that affect synapse numbers.

Interleukin-33 (IL-33) has also recently been identified to play an unexpected role in activity-dependent synaptogenesis in the adult hippocampus via microglia.[18] The authors showed that IL-33 is increased in the hippocampus in response to exposure to an enriched environment, which is known to increase neuronal activity.[18] They further showed that this experience-dependent production of neuronal IL-33 then stimulated microglia via the receptor IL1RL1 (also known as ST2) to induce genes related to phagocytosis and extracellular matrix (ECM) remodeling. Data further supported that neuronal IL-33/microglial IL1RL1 signaling promoted microglia to engulf and remodel the ECM around hippocampal synapses, which accommodated the formation of new synapses.

Similarly, another group showed that either 60-Hz light entrainment or ketamine exposure was sufficient for microglia to remove the ECM of perineuronal nets (PNNs) in the mouse cortex[23]; PNNs are composed of ECM molecules around inhibitory neurons and normally restrict plasticity.[24] Removal of PNNs by microglia, as mentioned above, resulted in enhanced cortical plasticity. Intriguingly, this PNN remodeling was dependent on neuron-IL-33→microglia-IL1RL1 signaling.[23] In addition to neurons, another study demonstrated that astrocytes can produce L-33 and induce synaptogenesis in the hippocampus.[25] However, unlike neurons, astrocytes were stimulated to produce IL-33 upon optogenetic or dark rearing–mediated silencing of hippocampal CA1 neurons. While silencing typically promoted a homeostatic increase in synapse numbers and spatial memory formation, this synaptogenic effect was blocked in mice in which astrocyte-derived IL-33 is ablated. It remains less clear if microglia carry out the synaptogenic function downstream of astrocytic IL-33–induced synaptogenesis, but pharmacologically blocking IL1RL1 resulted in a similar effect.

Intriguingly, in both studies above, increasing IL-33 induced synaptogenesis in the adult hippocampus but in different cell types and by seemingly opposing patterns of neural activity. That is, in one study,[18] elevating activity in the hippocampus with an enriched environment induced neuronal IL-33 production, whereas in the other study,[25] neuronal silencing induced astrocyte IL-33 production. Each of the studies used a different experimental paradigm to elicit elevated activity in the hippocampus, thus it could be that cell-type specific IL-33 production is sensitive to the pattern of activity. Although it is less

clear if neural activity is involved, another important consideration is that in a developmental context, astrocyte-derived IL-33 appears to induce microglial phagocytic transcriptional programs via IL1RL1/ST2 to facilitate microglial elimination of synapses[26–28] (Figure 1B). How would the same molecule elicit seemingly opposing effects on synapses depending on the age of the animal? It is possible that in both development and adult, phagocytic clearance programs are elicited in microglia by IL-33–IL1RL1 signaling, but other cues and receptors (e.g., complement, phosphatidyl serine [PtdSer], triggering receptor expressed on myeloid cells 2 [TREM2]) target microglia toward different phagocytic substrates. In development, for example, these cues could be enriched on synaptic membranes to elicit synapse elimination, whereas these cues also exist on ECM substrates in the adult brain to accommodate new synapses. Alternatively, mature neurons and microglia in the adult CNS may be transcriptomically or epigenetically programmed such that IL-33–IL1RL1/ST2 signaling elicits a different response compared to what occurs in the developing brain.

DEVELOPMENTAL SYNAPSE PRUNING DRIVEN BY SPONTANEOUS NEURAL ACTIVITY

While studies suggest that microglia perform an activity-dependent synaptogenic function, arguably their most widely studied function is in activity-dependent synaptic pruning. Synaptic pruning is a developmental process by which a subset of synapses is eliminated in the developing brain while other more active synapses are maintained, strengthened, and elaborated. In other words, pruning involves not only the elimination of some synapses, but also the stabilization of others in response to neuronal activity (Figure 1B).[29] Three independent groups showed that microglia can engulf and eliminate synaptic material during developmental synaptic pruning.[30–32] Below, we focus on studies that have shown that spontaneous neural activity, which occurs at early stages of circuit development prior to sensory experience, can drive microglial pruning function.

Data supporting the view that microglia respond to changes in spontaneous neural activity and subsequently prune synapses have been obtained from studies in the developing rodent retinogeniculate circuit. This circuit is composed of retinal ganglion cells (RGCs) that project their axons from the retina to the lateral geniculate nucleus (LGN) of the thalamus. It was first shown that complement proteins C1q and C3 localize to developing retinogeniculate synapses in the LGN, and that deletion of either of these molecules blocked the pruning of RGC presynaptic inputs.[33] Later, it was shown that retinogeniculate presynaptic inputs were engulfed and eliminated by microglia during pruning via complement C3 and its cognate receptor CR3 expressed by microglia.[30] Supporting an activity-dependent mechanism, when RGCs from one eye were silenced with tetrodotoxin, microglia preferentially engulfed presynaptic material within the LGN from those inactive neurons;[30] conversely, enhancing activity in a subset of neurons with a cyclic adenosine monophosphate analog decreased microglial engulfment of more active presynaptic inputs in the LGN. A later study showed that either pharmacologically blocking or genetically ablating adenosine A_{2A} receptors ($A_{2A}R$), which are known to drive retinal waves of spontaneous activity and retinogeniculate synaptic pruning,[34] can also result in decreased microglial engulfment and elimination of retinogeniculate synapses.[35] Despite this evidence for an activity-dependent mechanism, how activity influences microglial engulfment of synapses remained an open question.

More recently, activity-dependent exposure of PtdSer was implicated as the factor that modulates C1q binding to synapses and pruning by microglia.[36,37] Briefly, CDC50A (also known as TMEM30A), a chaperone of phospholipid flippases known to negatively regulate PtdSer exposure,[36] is decreased when neuronal activity decreases in cultured neurons. *Cdc50a* deletion in vivo was shown to increase PtdSer exposure at synapses and induce aberrant engulfment of synaptic material by microglia.[36,38] It is also worth mentioning that PtdSer can also bind other molecules on microglia to stimulate engulfment, such as TREM2, GPR56, and MERTK.[37–40] Thus, activity-dependent exposure of PtdSer could be important in both complement- and non-complement–dependent microglial pruning mechanisms.

Another group has shown that decreasing activity in a subset of neurons can induce JAK2–STAT1–dependent signaling in these neurons, and that JAK2–STAT1 signaling was required for callosal axon and cortical synapse pruning in the prefrontal cortex, as well as retinogeniculate synapse pruning.[41] It is possible that JAK2–STAT1 transcriptional programs elicit changes in PtdSer exposure in less active neurons, followed by microglial engulfment of synaptic material.

Another way in which activity can influence complement-dependent synaptic pruning in the retinogeniculate circuit is by modifying negative regulators of microglial synapse engulfment. For example, it has been shown that CD47–SIRPα signaling acts as an activity-dependent break on microglial engulfment of developing retinogeniculate presynaptic inputs.[42]

Besides mechanisms that directly modulate microglial engulfment of cellular substrates in the developing brain, it is also important to consider mechanisms by which microglia initially sense changes in activity (Figure 2). Early work suggested that a major microglial recruitment chemokine receptor, fractalkine receptor CX3CR1, is involved in recruiting microglia to hippocampal synapses undergoing pruning (Figure 2C).[31] Still, it was unclear if CX3CR1 signaling was neural activity dependent.

More recently, mechanisms have been identified by which microglia sense neural activity to regulate synapse pruning. For example, microglia sense global changes in neural network activity by sensing extracellular potassium via the potassium channel THIK-1.[43,44] Previous work showed that microglial surveillance of the brain parenchyma was dependent on expression of THIK-1[44] (Figure 2A). It was later shown that THIK-1–deficient mice had elevated synapse numbers in the developing hippocampus, concomitant with decreased engulfed presynaptic material within microglia.[43] The authors posited that this could be due to changes in the transcription of phagocytic genes in microglia in response to loss of THIK-1 and/or reduced surveillance by microglia in the THIK-1–deficient mice. Another possibility is that loss of THIK-1, and subsequent extracellular potassium

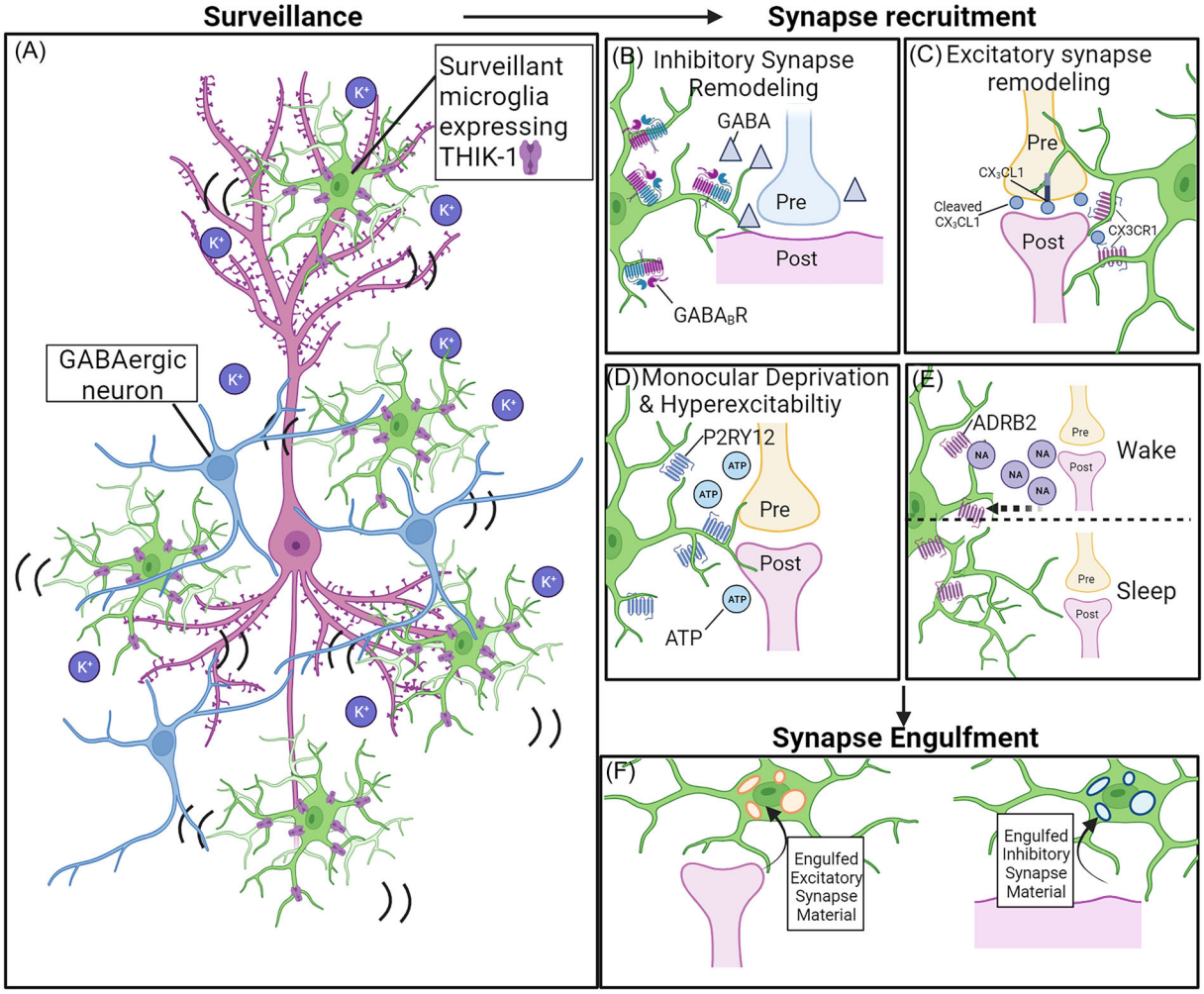

FIGURE 2 Activity-dependent factors that influence microglial surveillance and recruitment to synapses. (A) Microglia-expressed potassium channel THIK-1 regulates microglia motility and surveillance function, necessary for microglia to subsequently be recruited to and remodel synapses in response to activity. (B) Changes in neural activity during monocular deprivation and hyperactivity can result in heightened ATP release from neurons, which has been suggested to then recruit microglia to synapses to modulate their structure and function. (C) During hours of wakefulness, NA is higher in the cortex, resulting in decreased microglia–synapse contact through (ADRB2). In contrast, NA is lower during sleep resulting in enhanced microglia-synapse association. (D) Microglia also respond to GABAergic neurotransmission through $GABA_BR$ to modulate their contact with synapses and subsequently synapse remodeling. ADRB2, beta-2 adrenergic receptor; ATP, adenosine triphosphate; CX3CL1, fractalkine; CX3CR1, fractalkine receptor; GABA, gamma-aminobutyric acid; $GABA_BR$, GABA B receptor; NA, noradrenaline/norepinephrine; THIK-1, tandem pore domain halothane-inhibited potassium channel 1. Figure made with BioRender.

sensing by microglia, reduces the likelihood of microglia encountering a phagocytic substrate.

Another modulator of synapse surveillance by microglia is through direct sensing of neurotransmitters. This has been shown recently in the context of cortical development where a group identified a subpopulation of microglia in the somatosensory cortex that express $GABA_{B1}$ receptors ($GABA_{B1}R$). Ablation of this receptor specifically in microglia prevented engulfment and pruning of parvalbumin inhibitory synapses in the postnatal somatosensory cortex (Figure 2B).[45] This is in contrast to the study discussed above in which microglia appear to perform a synaptogenic role in a similar developmental window at inhibitory chandelier cell synapses in the somatosensory cortex through $GABA_{B1}R$.[16] These data suggest that GABA elicits a coordinated synaptogenic and synapse elimination function in microglia depending on the type of inhibitory neuron. It is also possible that microglia are locally heterogenous, and microglia surrounding different types of inhibitory neurons have differing responses to GABA. Additionally, in both contexts—-of GABA-mediated pruning in the cortex and THIK-1–mediated pruning in the hippocampus—-it remains unclear how microglia are physically engulfing synaptic material downstream of these pathways. Intriguingly, other work has implicated local externalized synaptic PtdSer[37] and TREM2[39] in developmental hippocampal and cortex synaptic pruning by regulating microglia-mediated synapse engulfment. Thus, it is likely that these mechanisms execute the synapse pruning function for microglia downstream of these ion- and neurotransmitter-sensing pathways (Figure 2F).

The studies discussed above demonstrate that microglia can respond to changes in spontaneous neural activity to prune developing

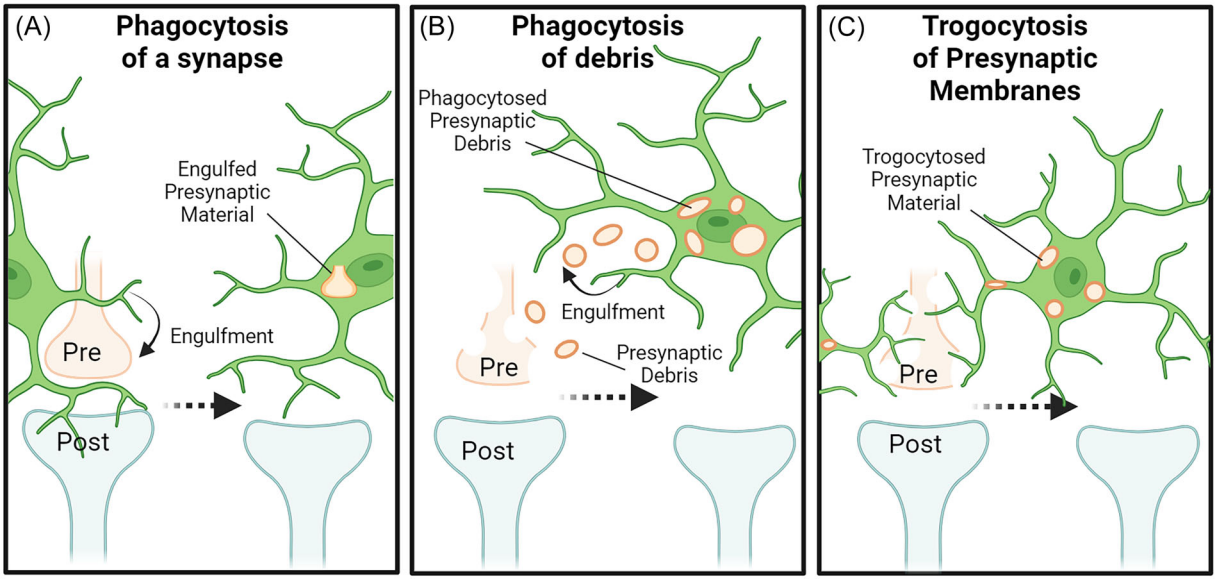

FIGURE 3 Putative mechanisms by which microglia engulf and remove synaptic material in response to changes in neural activity. (A) Microglia-mediated phagocytosis of an intact synaptic membrane. (B) Synapses degenerate and leave behind synaptic debris, which is then phagocytosed by microglia. (C) Microglia engulf synaptic membranes (trogocytosis), ultimately leading to synapse removal. Figure made with BioRender.

synapses through the engulfment of synaptic material. Still, it remains unclear if microglia carry out this engulfment activity by enveloping intact synapses followed by phagocytosis (Figure 3A). Alternatively, synaptic membranes could be shed by a neuron-autonomous mechanism followed by microglial engulfment (Figure 3B). Most recently, a trogocytosis-dependent mechanism has been suggested by live imaging studies in mouse hippocampal slices and in vivo in *Xenopus*.[46,47] These studies provided evidence that microglia preferentially trogocytose[a] presynaptic membranes and axons rather than engulfing whole synaptic compartments or postsynaptic material (Figure 3C). This is reminiscent of early work in mice showing that Schwann cells similarly engulf small parts of retracting motor neuron terminals and axons during neuromuscular junction pruning.[48]

Regardless of the exact mechanism of how microglia prune synaptic material, genetically blocking engulfment of synaptic material by microglia results in retention of structurally and functionally intact synapses, supporting an active role for microglia in the pruning process.

DEVELOPMENTAL SYNAPSE PRUNING DRIVEN BY EXPERIENCE-DRIVEN CHANGES IN NEURAL ACTIVITY

All the work described above focused on spontaneous neural activity driving microglia to engulf and prune synapses in early postnatal animals. However, sensory experience can also influence activity in a circuit to drive synapse pruning by either engulfment and/or synapse destabilization mechanisms (Figure 1B), particularly in later postnatal development. For example, dark rearing juvenile mice and then re-exposing them to light was shown to induce increased expression of the cytokine receptor fibroblast growth factor-inducible 14 (FN14) in LGN relay neurons and increased expression of the FN14 ligand TWEAK (TNF-associated weak inducer of apoptosis) in microglia.[49,50] The authors further found that synapse density was higher around microglia that did not express TWEAK in the LGN, and mice deficient in TWEAK had a defect in retinogeniculate synapse elimination in juvenile mice. How these synapses are destabilized and then physically eliminated in a postsynaptic FN14–microglia–TWEAK–dependent manner remains to be fully deciphered. However, data indicate this is likely through a neuron-autonomous disassembly of postsynaptic sites along LGN relay neurons.[29]

From retinogeniculate synapses, visual information from the retina is transmitted to the visual cortex. Early work showed by two-photon imaging in the juvenile mouse visual cortex that microglia increase their contact with layer II/III spines in the visual cortex in response to either dark adaptation or dark adaptation and re-exposure to light.[32] In the same study, there was also evidence of increased phagocytic inclusions within microglia in the visual cortex following dark adaptation, as well as re-exposure to light. More recently, the same group has worked toward a molecular mechanism by which changes in visual experience elicit microglia to engulf synapses within the visual cortex. Instead of dark adaptation, this study used monocular deprivation by suturing one eye closed during the developmental critical window of heightened plasticity in the mouse visual cortex.[51] Specifically, it was shown that monocular deprivation in juvenile mice resulted in microglia engulfment of synaptic material from excitatory synapses in the corresponding input-deprived primary visual cortex, and microglia engulfment was blocked in mice deficient in the purinergic receptor

[a] Greek: *trogo*; gnaw.

P2RY12. Additionally, P2RY12-deficient mice had a defect in synaptic plasticity (namely, ocular dominance plasticity) induced by monocular deprivation. The authors proposed that adenosine triphosphate (ATP) is released by neurons within the primary visual cortex in response to monocular deprivation, which then stimulates microglia recruitment to synapses via P2RY12 (Figure 2D). This mirrored earlier work in the microglia field that showed by two-photon live imaging that microglia respond and are recruited to sites of high ATP via expression of P2RY12.[2] Interestingly, another group used a pharmacological strategy to deplete microglia in the juvenile brain and showed heightened evoked neural activity in primary visual cortex and reduced orientation selectivity, but no change in ocular dominance plasticity in adult animals.[52] This later study suggested that microglia were remodeling inhibitory synapses, rather than excitatory synapses. It remains to be determined whether the data in these two studies are incongruent. That is, the neurons impacted by microglia appear to be different (excitatory vs. inhibitory neurons) and one study showed effects on ocular dominance plasticity, while the other did not. These opposing results may be explained by the timing of the manipulations (depletion of P2RY12 from birth versus depletion of microglia at P18) and/or the timing of the ocular dominance measurements (late juvenile vs. adult mice). In both studies, the mechanism by which microglia engulf synapses in the primary visual cortex was not identified; but unlike in the retinogeniculate circuit, it seems that complement may not be involved.[53]

In contrast, C1q and matrix metalloprotease 9 (MMP9) have been implicated in monocular deprivation–induced synapse engulfment and remodeling in the secondary visual cortex to regulate cross-modal plasticity in developing mice.[54] Cross-modal plasticity occurs when brain circuits reorganize to integrate functions of two or more sensory modalities, usually as a result of a decrease in a specific sensory input. In this study, the authors used monocular deprivation in late postnatal mice, which results in enhanced excitatory responses in the secondary visual cortex upon whisker stimulation.[54] The authors showed that this enhanced excitatory response in the secondary visual cortex upon whisker stimulation resulted from the removal of inhibitory synapses by microglia, as well as increased levels of C1q and MMP9 in the secondary visual cortex. Either pharmacological depletion of microglia or administration of a broad MMP inhibitor resulted in a failure to induce this cross-modal plasticity upon whisker stimulation. It remains to be tested whether blocking C1q has a similar effect, and whether C1q and MMP9 are mechanistically linked.

Another cortical area that has received attention in the context of experience-dependent microglial synaptic pruning by microglia is the barrel cortex. Specifically, when whiskers were removed from a postnatal mouse's snout, which is known to dampen activity in the corresponding barrel cortex, microglia were stimulated to engulf thalamocortical presynaptic inputs in layer IV of the deprived barrel cortex.[6] It was then shown that whisker deprivation induced transcription of the RNA encoding for the protease ADAM10 in neurons in the barrel cortex, a protease known to cleave the neuronal ligand fractalkine (CX3CL1) into a secreted form. Evidence suggests that CX3CL1 post-translationally modified to a secreted form then binds its receptor CX3CR1 on microglia to induce them to engulf synapses (Figure 1B). Importantly, in mice deficient in either CX3CR1 or CX3CL1, or upon pharmacological inhibition of ADAM10, thalamocortical synapse engulfment and elimination by microglia are completely blocked. As CX3CR1 is not an engulfment receptor, the mechanism downstream of this GPCR to elicit changes in engulfment remains an open question. One possibility is that this is through the recruitment of microglia to synapses (Figure 2C). Indeed, other work on the developing hippocampus and earlier postnatal barrel cortex showed a delay in the recruitment of microglia to synapse-dense regions.[31,55] However, in the barrel cortex, microglia numbers are similar in wild-type and CX3CR1/CX3CL1-deficient mice after whisker removal.[6] Alternatively, CX3CR1 could elicit downstream molecular changes in microglia making them more competent to engulf synapses.

Together, these data demonstrate that microglia can also respond to experience-driven changes in neural activity. An important consideration across both spontaneous and experience-driven microglia-mediated synaptic pruning is that different molecular mechanisms are used to execute this pruning, depending on the neuron type and brain region. For example, complement regulates the pruning activity of microglia in the retinogeniculate circuit and secondary visual cortex, but data suggest that it may not regulate the experience-dependent elimination of synapses in the barrel or primary visual cortices.[6,53,56] Likewise, CX3CR1 signaling regulates synapse pruning in the developing hippocampus and barrel cortex;[6,31] but it is not required for synapse elimination in the developing visual cortex following monocular deprivation.[57,58] One possibility is that neuronal diversity in molecular signatures and activity patterns drives differential expression of factors that drive this region-specific immune-mediated pruning.

It is also emerging that multiple mechanisms must coordinate to regulate synapse remodeling in a given circuit. This includes the orchestration of a combination of signals that regulate the motility or recruitment of microglia necessary to reach synaptic sites (e.g., by P2RY12 and THIK1) (Figure 2A–E), as well as mechanisms that regulate the physical engulfment of synaptic material (e.g., by complement) (Figures 1B and 2F).

ACTIVITY-DEPENDENT SYNAPSE ELIMINATION IN THE ADULT CNS

In addition to developmental synaptic pruning, there is evidence that microglia can also engulf and eliminate synapses in the adult brain through activity-dependent mechanisms in the contexts of learning and memory and sleep (Figures 1B and 2E). In the context of learning and memory, a role has emerged for microglia in the activity-dependent formation or stabilization of memory engram cells. Memory engram cells are groups of neurons that form and lose functional synaptic connections over the course of memory formation in an activity-dependent manner.[59,60] It was shown that either depleting microglia or inhibiting complement-dependent synapse engulfment prevented the loss of a previously formed memory by blocking the loss of synapses on engram cells in the hippocampus.[61]

Another context in which microglia have been shown to engulf and eliminate synapses in the adult brain in response to changes in neural activity is during sleep. In rat prefrontal cortex, for example, microglia were found by static imaging to be more phagocytic, and complement C3 was localized to more synapses early in the morning (zeitgeber time 0/ZT0), when the sleep phase begins for mice, compared to the beginning of the awake phase at ZT12.[62] As neural network activity is known to change over the sleep/wake cycle[63–65] and microglia engulfment of synapses in development has been shown to be activity-dependent in development,[6,30,32,51] this raises the possibility that microglia synapse engulfment and complement are modulated by changes in neural activity over the sleep/wake cycle in the adult brain. Supporting that this is an activity-dependent mechanism, noradrenaline, a key neuromodulator derived from neurons in the locus coeruleus to promote wakefulness throughout the brain,[64,65] is now appreciated to regulate microglial surveillance and engulfment of synapses in the cortex over the sleep/wake cycle (Figure 2E). Specifically, microglial phagocytosis and sleep were enhanced in the rat prefrontal cortex by the application of an agent to deplete monoamines, including noradrenaline.[62] Intriguingly, the same study showed that noradrenaline applied directly to microglia *in vitro* resulted in reduced phagocytosis.[62] Other groups have shown that cortical microglia, which express β_2-adrenergic receptors, respond to noradrenaline by reducing their surveillance activity during wakefulness.[66,67] Likewise, microglia increase their surveillance and interaction with synapses during sleep in response to activity-dependent decreases in noradrenaline (Figure 2E).

Together, the above studies demonstrate that neuronal activity, via modulation of complement, can influence microglia-mediated elimination of synapses in the adult brain, which may be an important aspect of lifelong synapse plasticity and homeostasis. Precisely how changes in neural activity modulates complement remains a key open question. It also remains unknown how β_2-adrenergic receptor signaling in microglia modulates their surveillance and engulfment functions over the sleep/wake cycle.

Besides neural activity, another possible mechanism for microglia synapse pruning is by gene regulation of microglial motility and phagocytic function from circadian rhythm-regulated transcriptomic programs (Figure 1B), which rhythmically change over the course of 24 hours. This was recently supported by work in the adult hippocampus showing that microglia-dependent synapse phagocytosis and complement were increased upon deletion of the gene expressing REV-ERBα (*Nr1d1*), a transcriptional repressor downstream of the master clock protein BMAL1.[68] Interesting, deleting the master clock gene *Bmal1* only in CD11b-expressing myeloid cells, including microglia, in aged mice resulted in the opposite result, that is decreased complement deposition, decreased microglia engulfment of synapses, and increased immature dendritic spines in the hippocampus.[69] These seemingly opposing results may be a consequence of differences in global versus cell-specific ($CD11b^+$ cell) deletion. Alternatively, aging could result in changes in the molecular signature of cells that subsequently affects the outcome of *Bmal1* deletion. Testing these possibilities would require a side-by-side comparison of *Bmal1* and/or *Nr1d1* deletion in young versus aged mice, as well as a comparison of global versus cell-specific deletion. It will also be important to better understand the potential intersection of activity-dependent mechanisms governing sleep/wake and the clock. It is possible that the dominant mechanisms governing microglia over 24 h are region dependent. For example, sleep/wake-promoting changes in neural activity governing cortical microglia function could result from the abundant locus coeruleus noradrenergic projections in the cortex,[66] whereas circadian clock genes may have a stronger influence in subcortical structures.

ACTIVITY-DEPENDENT MECHANISMS TO MODULATE FUNCTIONAL CONNECTIVITY

All the studies described above demonstrated roles for microglia in the activity-dependent regulation of synapse numbers. It is also relevant to consider emerging work demonstrating that microglia can tune circuit function—perhaps without changing structural synapse numbers—in an activity-dependent manner (Figure 1C,D). In this context, microglia-dependent regulation of circuit function in response to purinergic signaling has been most widely studied. Live imaging studies first demonstrated that microglia respond robustly to ATP and direct their process motility toward sites of higher ATP.[2,3,70,71] This work showed that microglial processes converge on synaptic sites following NMDA receptor–mediated stimulation of neurons in an ATP-dependent manner.[70,71] It was further shown that microglia detected neuron-derived ATP gradients largely through the microglia-expressed purinergic receptor P2RY12.[72] Toward a functional relevance for these ATP-dependent changes in microglia-synapse contacts, it was identified by live imaging in the adult mouse cortex that individual synapses increase calcium during microglia contact.[73] Importantly, elevations in synaptic calcium upon microglia contact were decreased after treating mice with lipopolysaccharide, which also resulted in decreased overall synchronization of neural activity. Intriguingly, in separate studies, activity-dependent microglia–neuronal soma contacts served an opposite purpose, to decrease neural activity. In zebrafish, when neurons increased activity, there was an increased ATP-dependent association of microglia processes with neuronal soma, which was followed by a decrease in neural activity.[74] A similar result was observed in the mouse cortex.[75] It was further shown in the mouse study that without P2RY12 there was increased global neuronal calcium flux and increased excitotoxic injury in an ischemia model.[75] These studies demonstrated that microglia play important roles in monitoring neural activity and suggested that microglia could serve to quiet hyperactive neurons through purinergic signaling (Figure 1C). This was further supported by studies that showed increased neural activity and/or seizures in mice lacking microglia, G_i signaling in microglia, or P2RY12.[7,76–78] The suppressive effect of P2RY12 activity in microglia on neural activity was later elegantly demonstrated to result from P2RY12-mediated recruitment of microglial processes to hyperactive neurons followed by hydrolysis of ATP/ADP to AMP by CD39 expressed on the surface of microglia. The authors provided further evidence indicating that AMP is subsequently converted to adenosine, likely through CD73 on

microglia. Adenosine, in turn, suppressed striatal D1 neural activity through adenosine A1 receptor (A1R).[7] A similar P2RY12-dependent regulation of adenosine levels has also been recently implicated in regulating norepinephrine and promoting sleep.[79] One important open question is whether adenosine is acting directly on neurons or rather on astrocytes, which also express adenosine receptors and have key roles in regulating synaptic activity.

Besides ATP-dependent mechanisms, microglia-derived cytokines are emerging as potent neuromodulators.[80] As many CNS cells can produce these cytokines in health and disease, we restrict our discussion here to microglia-specific effects in the healthy CNS. One microglia-derived cytokine that has been shown to regulate circuit function in response to changes in activity is TNFα. During times of prolonged heightened neural activity or prolonged quieting of neuronal firing, neuron numbers increase or decrease, respectively, the amount of neurotransmitter receptors expressed in the postsynaptic membrane (Figure 1D).[81] It is now appreciated that microglia contribute to this form of plasticity called *synaptic* or *homeostatic scaling* by releasing TNFα that leads to scaling up and down of synaptic AMPA and $GABA_A$ receptors.[82-85] It was further shown that seizures could be exacerbated by microglia-derived TNFα, and that blocking brain-derived TNFα reduced seizure severity.[86,87] These data raise the possibility that microglia are responding to large changes in neural network activity by releasing TNFα to modulate synaptic plasticity and function. Similar to mice either lacking P2RY12 or with heightened TNFα, CX3CR1-deficient mice have increased seizure phenotypes.[88] It remains to be determined how CX3CR1 is mediating this effect, but it is intriguing to consider that previously discussed activity-dependent developmental mechanisms that engage CX3CR1–CX3CL1 signaling to regulate synapse numbers[6,31] may be involved. This is supported by another mechanism in which experimentally increasing microglia-mediated synapse engulfment in the mouse hippocampus by increasing PtdSer exposure results in elevated seizures.[38]

CONCLUSIONS

The field of microglia research has progressed exponentially, and microglia are emerging as key components of neural circuits. Microglia are not simply bystanders but actively responding to neural networks via neurotransmitters, purinergic signaling, and activity-dependent regulation of immune molecules. In turn, microglia translate changes in neural activity to shape synaptic connectivity and function in both development and adulthood.

Several different immune-related mechanisms, such as fractalkine and complement, have now been identified to regulate activity-dependent synapse remodeling. However, it is less clear why this immune signaling appears to be neuron and/or brain region dependent. One possibility is that this diverse immune signaling results from neuronal diversity and/or differences in firing that differentially regulate immune signaling within a circuit.

Similarly, it remains unclear how mechanisms that regulate microglial motility and recruitment may coordinate with engulfment mechanisms to regulate neural networks. Also, microglia appear to use purinergic signaling, as well as neurotransmitter and cytokine signaling, to regulate the excitability of neurons. Do these mechanisms act together or are they, again, neuron/circuit-specific?

Also, it is important to better understand how neural activity drives changes in immune-related molecules, which subsequently modulate microglia function in circuits.

Finally, most work has focused on specific activities of microglia on neurons, but it is less appreciated how astrocytes, as well as other resident CNS cell types, may serve as intermediaries in the activity-dependent regulation of synapses by microglia. Studies highlighted in our review and which prompt such open questions relied on either microglia-specific depletion strategies (genetic and pharmacological) or microglia-specific inducible CreER lines. While these recent tools have helped the field grow significantly over the past decade, they lack the level of specificity and/or efficiency required to definitively answer some of the questions we have discussed above. Microglia depletion strategies are not microglia-specific and impact brain border macrophages and peripheral immune cell populations.[89-92] Microglia-specific inducible CRE lines similarly are not uniquely microglia specific and, depending on the specific gene targeted, the efficiency of CRE-mediated excision can be relatively low.[93] Development of better, more efficient technologies will likely uncover additional insights and answer some of the ones we have raised in our discussion here. Manipulation of microglia more directly and locally, without the need for breeding multiple mouse lines, is key. This would allow the field to evaluate different molecular pathways rapidly and in a neuron and/or circuit-specific way. The field is beginning to make headway on specifically targeting microglia with new adeno-associated viruses vectors that are more tropic for microglia, though this will require further testing.[94,95]

In summary, microglia receive and translate activity-dependent neuronal signals, including signals by neurotransmitters, ATP, and immune molecules, to intimately associate with and fine-tune synaptic connectivity. The field is now beginning to understand vital functions for microglia within neural circuits, which will benefit from further development of more specific tools to manipulate microglia. Advances will change the way we understand neuronal and circuit functions, and they will likely have important implications for understanding mechanisms of circuit dysfunction in human brain diseases.

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V.D.L. and D.P.S. wrote and edited the manuscript.

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COMPETING INTERESTS

The authors declare no competing interests.

ORCID

Dorothy P. Schafer https://orcid.org/0000-0003-2201-6276

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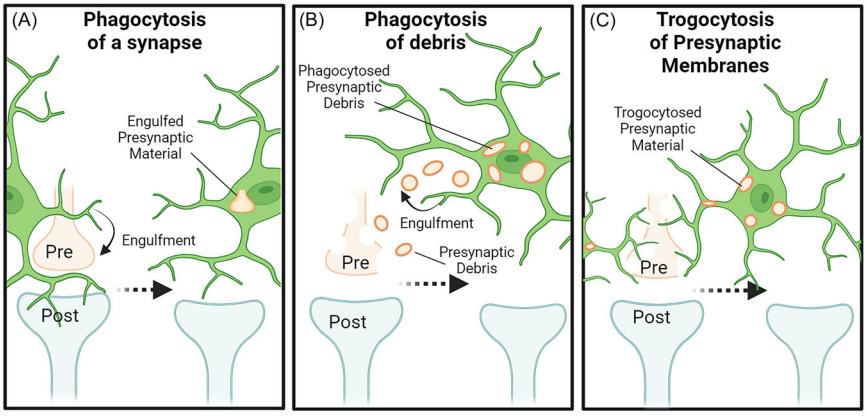

In this review, we highlight current data supporting that microglia are continuously monitoring and responding to neural activity to then modulate synapse structure and function.